

Appl. No. 09/808,706  
Amtd. dated August 13, 2003  
Reply to Office Action of February 13, 2003

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This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Please cancel claims 9-28 without prejudice as being drawn to non-elected species of invention; amend claim 1; and add new claims 29-51 as follows:

1. (currently amended) A method for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the method comprising the steps of:
  - a) amplifying the unknown starting quantity of the target nucleic acid sequence in the test sample;
  - b) amplifying a plurality of known starting quantities of a calibration nucleic acid sequence in respective calibration samples;
  - c) determining a respective threshold value for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples and for the target nucleic acid sequence in the test sample, wherein the threshold value is determined for each nucleic acid sequence in a respective sample by:
    - i) measuring, at a plurality of different times during amplification, at least one signal whose intensity is related to the quantity of the nucleic acid sequence being amplified in the sample;
    - ii) deriving a growth curve from the measurements of the signal;
    - iii) calculating a derivative of the growth curve;
    - iv) identifying a characteristic of the derivative; and
    - v) determining a threshold value associated with the characteristic of the derivative;
  - d) deriving a calibration curve from the threshold values determined for the known starting quantities of the calibration nucleic acid sequence in the calibration samples; and

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- e) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.
- 2. (original) The method of claim 1, wherein each of the threshold values comprises a cycle number.
- 3. (original) The method of claim 1, wherein each of the threshold values comprises an elapsed time of amplification.
- 4. (original) The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.
- 5. (original) The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a negative peak of the second derivative.
- 6. (original) The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.
- 7. (original) The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.
- 8. (original) The method of claim 1, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the step of determining the threshold value associated with the positive peak comprises:
  - i) fitting a second order curve to the second derivative data points; and
  - ii) calculating the threshold value as the location of a peak of the second order curve.
- 9-28. (canceled)

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29. (new) A method for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the method comprising the steps of:

- a) amplifying the unknown starting quantity of the target nucleic acid sequence in the test sample;
- b) amplifying a plurality of known starting quantities of a calibration nucleic acid sequence in respective calibration samples;
- c) determining a respective threshold value for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples and for the target nucleic acid sequence in the test sample, wherein the threshold value is determined for each nucleic acid sequence in a respective sample by:
  - 1) measuring, at a plurality of different times during amplification, at least one signal whose intensity is related to the quantity of the nucleic acid sequence being amplified in the sample;
  - 2) storing signal values defining a growth curve for the nucleic acid sequence, wherein the growth curve expresses signal intensity as a function of cycle number or as a function of time of amplification;
  - 3) determining a derivative of the growth curve, wherein the derivative is determined with respect to cycle number or time; and
  - 4) calculating a threshold cycle number or time value associated with a characteristic of the derivative;
- d) deriving a calibration curve from the threshold values determined for the known starting quantities of the calibration nucleic acid sequence in the calibration samples; and
- e) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.

30. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the second derivative of the growth curve with respect to cycle number and calculating the threshold cycle number as the location, in cycles, of a maximum of the second derivative.

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31. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the second derivative of the growth curve with respect to cycle number and calculating the threshold cycle number as the location, in cycles, of a minimum of the second derivative.
32. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the second derivative of the growth curve with respect to cycle number and calculating the threshold cycle number as the location, in cycles, of a zero-crossing of the second derivative.
33. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the first derivative of the growth curve with respect to cycle number and calculating the threshold cycle number as the location, in cycles, of a maximum of the first derivative.
34. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises a maximum of the second derivative, and wherein steps (c3) and (c4) comprise:  
calculating second derivative values of the growth curve, with respect to cycle number, at a number of different measurement points to yield a plurality of second derivative data points;  
fitting a second curve to at least three of the second derivative data points; and  
calculating the threshold cycle number as the location, in cycles, of a positive peak of the second curve.
35. (new) The apparatus of claim 34, wherein the threshold cycle number at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.
36. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises a minimum of the second derivative, and wherein steps (c3) and (c4) comprise:  
calculating second derivative values of the growth curve, with respect to cycle number, at a number of different measurement points to yield a plurality of second derivative data points;  
fitting a second curve to at least three of the second derivative data points; and  
calculating the threshold cycle number as the location, in cycles, of a negative peak of the second curve.

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37. (new) The apparatus of claim 36, wherein the threshold cycle number at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.
38. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises a maximum of the first derivative, and wherein steps (c3) and (c4) comprise: calculating first derivative values of the growth curve, with respect to cycle number, at a number of different measurement points to yield a plurality of first derivative data points; fitting a second curve to at least three of the first derivative data points; and calculating the threshold cycle number as the location, in cycles, of a peak of the second curve.
39. (new) The apparatus of claim 38, wherein the threshold cycle number at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three first derivative data points.
40. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises a zero-crossing of the second derivative, and wherein steps (c3) and (c4) comprise: calculating second derivative values of the growth curve, with respect to cycle number, at a number of different measurement points to yield a plurality of second derivative data points; and calculating the threshold cycle number at the zero-crossing by interpolation between at least two of the second derivative data points.
41. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the second derivative of the growth curve with respect to time of amplification and calculating the threshold time value as the location, in time of amplification, of a maximum of the second derivative.
42. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the second derivative of the growth curve with respect to time of amplification and calculating the threshold time value as the location, in time of amplification, of a minimum of the second derivative.

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43. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the second derivative of the growth curve with respect to time of amplification and calculating the threshold time value as the location, in time of amplification, of a zero-crossing of the second derivative.
44. (new) The apparatus of claim 29, wherein steps (c3) and (c4) comprise determining the first derivative of the growth curve with respect to time of amplification and calculating the threshold time value as the location, in time of amplification, of a maximum of the first derivative.
45. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises a maximum of the second derivative, and wherein steps (c3) and (c4) comprise:  
calculating second derivative values of the growth curve, with respect to time of amplification, at a number of different measurement points to yield a plurality of second derivative data points;  
fitting a second curve to at least three of the second derivative data points; and  
calculating the threshold time value as the location, in time of amplification, of a positive peak of the second curve.
46. (new) The apparatus of claim 45, wherein the threshold time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.
47. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises a minimum of the second derivative, and wherein steps (c3) and (c4) comprise:  
calculating second derivative values of the growth curve, with respect to time of amplification, at a number of different measurement points to yield a plurality of second derivative data points;  
fitting a second curve to at least three of the second derivative data points; and  
calculating the threshold time value as the location, in time of amplification, of a negative peak of the second curve.

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48. (new) The apparatus of claim 47, wherein the threshold time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.
49. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises a maximum of the first derivative, and wherein steps (c3) and (c4) comprise:
  - calculating first derivative values of the growth curve, with respect to time of amplification, at a number of different measurement points to yield a plurality of first derivative data points;
  - fitting a second curve to at least three of the first derivative data points; and
  - calculating the threshold time value as the location, in time of amplification, of a peak of the second curve.
50. (new) The apparatus of claim 49, wherein the threshold time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three first derivative data points.
51. (new) The apparatus of claim 29, wherein the characteristic of the derivative comprises zero-crossing of the second derivative, and wherein steps (c3) and (c4) comprise:
  - calculating second derivative values of the growth curve, with respect to time of amplification, at a number of different measurement points to yield a plurality of second derivative data points; and
  - calculating the threshold time value at the zero-crossing by interpolation between at least two of the second derivative data points.